Barry R Masters, Peter T C So, and Enrico Gratton.

Multi-photon excitation fluorescence microscopy and spectroscopy of in vivo human skin.

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Abstract

Multi-photon excitation microscopy at 730 nm and 960 nm was used to image the autofluorescence of in vivo human skin from the surface to a depth of about 200 microns. The emission spectra and fluorescence lifetimes were determined. We have imaged the fluorescent cytoplasm of individual skin cells at depths of between 25 to 75 µm below the skin surface for both excitation wavelengths. Fluorescence emission spectra and fluorescent lifetimes of the tissue were obtained at single points, relative to the surface (0-50 microns) and at deeper depths (100-150 microns). The source of the fluorescence emission spectra and fluorescence lifetimes are consistent with reduced pyridine nucleotides, NAD(P)H, as the primary source of the skin autofluorescence using 730 nm excitation. With 960 nm excitation, fluorescence emission at 520 nm indicates the presence of flavoprotein contribution. A second, discrete fluorescence emission component, which starts at 425 nm is observed with 960 nm excitation. This presence of fluorescence emission at wavelengths less than half the excitation wavelength suggests three or more photon excitation processes. This conjecture is further confined by the observation that fluorescence intensity increases with excitation intensity at a power of 2.5. Further work is required to identify these emitting species. This study demonstrates the use of multiphoton excitation microscopy for functional imaging of the metabolic states of in viva human skin cells. (This work was supported by NIH RRO3 155.)